PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference TIE-001-PCT-PRIO1	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP2003/009393	International filing date *(day/month/year)* 25.08.2003	Priority date *(day/month/year)* 25.08.2003
International Patent Classification (IPC) or both national classification and IPC G01N33/543		
Applicant RAMAEL, Marc, et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 8 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
25.03.2005	23.12.2005
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP2003/009393

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-66 as originally filed

Claims, Numbers

1-33 received on 12.12.2005 with letter of 09.12.2005

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 13, 30 (both partially)

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. as above.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-33
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-33
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-33
	No:	Claims	

2. Citations and explanations

   see separate sheet

Section III:

1. The subject-matter of claims 13 and 30 has only partially been searched (see International Search Report). Consequently, no opinion will be established on subject-matter encompassed by said claims but which has not been searched (Rule 66.1(e) PCT).

Section V:

The following document, cited in the International search report, is referred to in this International Preliminary Examination Report:

D1: TORCHILIN V P ET AL: "ANTIBODY-LINKED CHELATING POLYMERS FOR IMMUNOIMAGING IN VIVO" JOURNAL OF CONTROLLED RELEASE, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, NL, vol. 11, 1989, pages 297-303.

1. D1 discloses antibody-linked chelating polymers for immunoimaging, in particular (see abstract) dextran derivatives comprising antibodies to analyte. This derivative may be further modified with biotin (which is bound either to dextran or antibody).
D1 also discloses antibody-polymer conjugates wherein the polymer possesses large numbers of metal binding sites (see p.297, left-hand column, second sentence) and p.300, right-hand column, last sentence to p.301, left-hand column, first sentence).
Secondary labelled antibody is added after the conjugate is added to the sample.

Present claim 1 is distinguished therefrom by the following features: Addition of antibody against the tag on the analyte, the antibody having a metal particle label of average diameter between 0.6 and 40 nm; Addition of antibody conjugate comprising one or

more anti-A antibodies directed against immunoglobulins of species A and one or more anti-B antibodies directed against immunoglobulins of species B; A metal enhancement step.
Therefore, the subject-matter of claim 1 complies with the requirements of Article 33(2) PCT.

The technical problem to be solved by present claim 1 with respect to the method disclosed in D1 is the provision of a sensitive in vitro method with reduced background signal.

The solution defined in claim 1 is not suggest in any of the available documents taken alone or in combination. Therefore, the subject-matter of claim 1 seems to comply with the requirements of Article 33(3) PCT.
The same reasoning applies to independent claims 2 to 5 and 23 to 26, which also comprise the features use of a metal particle label of average diameter between 0.6 and 40 nm; Addition of antibody conjugate comprising one or more anti-A antibodies directed against immunoglobulins of species A and one or more anti-B antibodies directed against immunoglobulins of species B; A metal enhancement step.

Claims 6 to 9, 27 and 31 to 33 refer back to said independent claims and therefore also comply with the requirements of Article 33(2) and (3) PCT.

None of the available prior art documents discloses a conjugate comprising one or more antibodies against immunoglobulins of species A, one or more antibodies against immunoglobulins of species B and metal particles of average diameter between 0.6 and 40 nm. Therefore, the subject-matter of claims 10 to 22 to 28 to 30 is novel (Article 33(2) PCT).
For the same reasoning as above the solution defined in independent claims 10 and 28 seems to involve an inventive step. Therefore, the subject-matter of claims 10 and 28, and dependent claims 11 to 22, 29 and 30 appears to comply with the requirements of Article 33(3) PCT.

2. The subject-matter of claims 1 to 33 is industrially applicable.

CLAIMS (RETYPED)

1. A method for quantitatively and/or qualitatively detecting one or more components in one or more samples, said component capable of binding to a probe, comprising the steps in the following order:

a) applying one or more samples onto a solid support,

b) optionally storing solid support of step a) at a temperature between 0 and 10 degrees Celsius,

c) incubating solid support of step a) or b) with one or more tagged probes,

d) incubating solid support with a monoclonal or polyclonal antibody directed against the tag of step c), said antibody raised in species A and said antibody labelled with metal particles of average diameter between 0.6 nm and 40nm,

e) incubating solid support with antibody conjugate, said conjugate comprising:

- one or more antibodies, anti-A, directed against immunoglobulins of species A,
- one or more antibodies, anti-B, directed against immunoglobulins of species B,
- optionally one or more substances which directly or indirectly cause a quantitative colour change compared with the solid support,

f) incubating the solid support with a polypeptide capable recognition by anti-B antibodies, said polypeptide labelled with one or more substances which directly or indirectly cause a quantitative colour change compared with the solid support, and

g) incubating the solid support with a metal enhancement reagent

and optionally with a colour change reagent that is a suitable substrate of an enzyme attached to the antibody conjugate, and

h) reading the solid support to quantitatively and/or qualitatively detect said components.

2. A method for quantitatively and/or qualitatively detecting one or more components in one or more samples, said component capable of binding to a probe, comprising the steps in the following order:

a) applying one or more samples onto a solid support,

b) optionally storing solid support of step a) at a temperature between 0 and 10 degrees Celsius,

c) incubating solid support of step a) or b) with one or more tagged probes,

d) incubating solid support with a monoclonal or polyclonal antibody directed against the tag of step c), said antibody raised in species A and said antibody optionally labelled with metal particle,

e) incubating solid support with antibody conjugate, said conjugate comprising:

- one or more antibodies, anti-A, directed against immunoglobulins of species A,
- one or more antibodies, anti-B, directed against immunoglobulins of species B,
- metal particles of average diameter between 0.6 nm and 40nm,

f) incubating the solid support with a polypeptide capable recognition by anti-B antibodies, said polypeptide labelled with one or more substances which directly or indirectly cause a quantitative colour change compared with the solid support, and

g) incubating the solid support with a metal enhancement reagent and optionally with a colour change reagent that is a suitable substrate of an enzyme attached to the antibody conjugate, and

h) reading the solid support to quantitatively and/or qualitatively detect said components.

3. A method for quantitatively and/or qualitatively detecting one or more components in one or more samples, said component capable of binding to a probe, comprising the steps in the following order:

a) applying one or more samples onto a solid support,

b) optionally storing solid support of step a) at a temperature between 0 and 10 degrees Celsius,

c) incubating solid support of step a) or b) with one or more tagged probes,

d) incubating solid support with a monoclonal or polyclonal antibody directed against the tag of step c), said antibody raised in species A and said antibody optionally labelled with metal particle,

e) incubating solid support with antibody conjugate, said conjugate comprising:

- one or more antibodies, anti-A, directed against immunoglobulins of species A,
- one or more antibodies, anti-B, directed against immunoglobulins of species B,
- optionally one or more substances which directly or indirectly cause a quantitative colour change compared with the solid support,

f) incubating the solid support with a polypeptide capable recognition by anti-B antibodies, said polypeptide labelled with metal particles of average diameter between 0.6 nm and 40nm, and

g) incubating the solid support with a metal enhancement reagent and optionally with a colour change reagent that is a suitable substrate of an enzyme attached to the antibody conjugate, and

h) reading the solid support to quantitatively and/or qualitatively detect said components.

4. A method for quantitatively and/or qualitatively detecting one or more components in one or more samples, said component capable of binding to a probe, comprising the steps as defined in any of one of claims 1 to 3 wherein step a) is

a) applying one or more probes onto a solid support,

and step c) is

c) incubating solid supports with tag-labelled sample,

5. A method for quantitatively and/or qualitatively detecting one or more components in one or more samples, said component capable of binding to a probe, comprising the steps as defined in any one of claims 1 to 4 wherein step c) is absent and step d) is

d) incubating solid supports with metal-particle-labelled anti-component monoclonal or polyclonal antibody, said antibody raised in species A.

6. A method according to any of claims 1 to 5 further comprising the steps, after step f), of:

f-1) repeating steps e) to f), and

f-2) optionally repeating step f-1).

7. A method according to any of claims 4 to 6 wherein the solid support is supplied with probe pre-applied, and step a) is not performed by the user.

8. A method according to any of claims 1 to 7 wherein the reading of step h) comprises the use of a colour chart.

9. A method according to any of claims 1 to 7 wherein the reading of step h) comprises the use of a device suitable for detecting changes in conductance and/or current across the solid support at the positions at which said samples are applied.

10. A kit for quantitatively and/or qualitatively detecting one or more components in one or more samples, said component capable of binding to a probe, comprising:

a) one or more solid supports,

b) a container in which a quantity antibody conjugate is present, said conjugate comprising:

- one or more antibodies, anti-A, directed against immunoglobulins of species A,
- one or more antibodies, anti-B, directed against immunoglobulins of species B,
- metal particles of average diameter between 0.6 nm and 40nm.

11. A kit according to claim 10 further comprising a container in which a quantity of anti-tag polyclonal or monoclonal antibodies is present, said antibodies raised in species A.

12. A kit according to claims 10 or 11 wherein the solid support is pre-loaded with probes capable of binding to said components.

13. A kit according to any of claims 10 to 12 for use in a method of claims 1 to 9.

14. A kit according to any of claims 10 to 13 for use in detecting, diagnosing and/or monitoring the progress of a Human Papillomavirus (HPV) infection and wherein one or more molecular probes is capable of binding to an HPV component.

15. A kit according to claim 14 wherein said component is a coat polypeptide.

16. A kit according to claim 15 wherein said component is a gene selected from the group consisting of HPV 16, HPV18, HPV 31, HPV 33, HPV 35, HPV 52 and HPV 58.

17. A kit according to any of claims 10 to 13 for use in detecting, diagnosing and/or monitoring the progress of one or more of the disease states in humans as listed in Table 1, by detecting a polypeptide and/or nucleic acid corresponding to the listed component.

18. A kit according to any of claims 10 to 13 for use in detecting, diagnosing and/or monitoring the progress infections caused by one or more of one or more of HCV, HIV, HBV, HTLV, mycobacteria, *Staphylococcus aureus.*

19. A kit according to any of claims 10 to 13 for use in detecting, diagnosing and/or monitoring the progress neurodegenerative diseases by detecting one or more of beta-amyloids, hTAU, phosphoTAU and APOE.

20. A kit according to any of claims 10 to 13 for use in detecting, diagnosing and/or monitoring the progress of malignant diseases, autoimmunity or allergy related diseases by detecting one or more of ANA, Jo-1, Myeloperoxidase, RNP, Scl-70, Sm, SS-A, IgE, IgG-subclasses and circulating antibodies.

21. A kit according to any of claims 10 to 13 for use in environmental testing of water for bacteria.

22. A kit according to any of claims 10 to 13 for use in environmental testing of food components for genetically modified components, listeria and salmonella.

23. A method for staining components in cell and/or tissue sections suitable for visualisation using microscopy comprising the steps of:

a) incubating said section with one or more tagged probes directed against a component,

b) incubating said section with metal labelled anti-tag monoclonal or polyclonal antibody, said antibody raised in species A, where the metal is particles of average diameter between 0.6 nm and 40nm,

c) incubating said section with antibody conjugate, said conjugate comprising at least:

- one or more antibodies, anti-A, directed against immunoglobulins of species A,
- one or more antibodies, anti-B, directed against immunoglobulins of species B,
- optionally one or more substances which directly or indirectly cause a quantitative colour change,

d) incubating the section with a polypeptide capable recognition by anti-B antibodies, said polypeptide labelled with one or more substances which directly or indirectly cause a quantitative colour change, and

e) incubating the section with a metal enhancement reagent and optionally with a colour change reagent that is a suitable substrate of an enzyme attached to the antibody conjugate.

24. A method for staining components in cell and/or tissue sections suitable for visualisation using microscopy comprising the steps of:

a) incubating said section with one or more tagged probes directed against a component,

b) incubating said section with metal labelled anti-tag monoclonal or polyclonal antibody, said antibody raised in species A,

c) incubating said section with antibody conjugate, said conjugate comprising at least:

- one or more antibodies, anti-A, directed against immunoglobulins of species A,
- one or more antibodies, anti-B, directed against immunoglobulins of species B,
- metal particles of average diameter between 0.6 nm and 40nm,

d) incubating the section with a polypeptide capable recognition by anti-B antibodies, said polypeptide labelled with one or more substances which directly or indirectly cause a quantitative colour change, and

e) incubating the section with a metal enhancement reagent and optionally with a colour change reagent that is a suitable substrate of an enzyme attached to the antibody conjugate.

25. A method for staining components in cell and/or tissue sections suitable for visualisation using microscopy comprising the steps of:

a) incubating said section with one or more tagged probes directed against a component,

b) incubating said section with metal labelled anti-tag monoclonal or polyclonal antibody, said antibody raised in species A,

c) incubating said section with antibody conjugate, said conjugate comprising at least:

- one or more antibodies, anti-A, directed against immunoglobulins of species A,
- one or more antibodies, anti-B, directed against immunoglobulins of species B,
- optionally one or more substances which directly or indirectly cause a quantitative colour change,

d) incubating the section with a polypeptide capable recognition by anti-B antibodies, said polypeptide labelled with metal particles of average diameter between 0.6 nm and 40nm, and

e) incubating the section with a metal enhancement reagent and optionally with a colour change reagent that is a suitable substrate of an enzyme attached to the antibody conjugate.

26. A method for staining components in cell and/or tissue sections suitable for visualisation using microscopy comprising the steps as defined in any of claims 23 to 25 wherein step a) is absent and step b) is

b) incubating section with metal particle labelled anti-component monoclonal or polyclonal antibody, said antibody raised in species A.

27. A method according to any of claims 23 to 26 further comprising the steps, after step d), of:

d-1) repeating steps c) to d), and

d-2) optionally repeating step d-1).

28. A kit for staining components in cell and/or tissue sections suitable for visualisation using microscopy comprising:

a container in which a quantity of antibody conjugate, said conjugate comprising at least:

- one or more antibodies, anti-A, directed against immunoglobulins of species A,
- one or more antibodies, anti-B, directed against immunoglobulins of species B,
- metal particles of average diameter between 0.6 nm and 40nm.

29. A kit according to claim 28 further comprising a container in which a quantity of anti-tag polyclonal or monoclonal antibodies is present, said antibodies raised in species A.

30. A kit according to claim 28 or 29 for use in a method of any of claims 23 to 27.

31. A method according to any of claims 1 to 9, 23 to 27, and a kit according to any of claims 10 to 22 and 28 to 20 wherein said metal particle is gold.

32. A method according to any of claims 1 to 9, 23 to 27, and a kit according to any of claims 10 to 22 and 28 to 20 wherein said tag is biotin.

33. A method according to any of claims 1 to 9, 23 to 27, and a kit according to any of claims 10 to 22 and 28 to 20 wherein said polypeptide capable recognition by anti-B antibodies is labelled with gold particles and/or alkaline phosphatase.